Sulfur metabolism, glucosinolates and fungal resistance in Brassica

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Chapter 5

Infection of *Brassica rapa* with necrotrophic fungi enhances the content of indolic glucosinolates

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Abstract

Brassica species contain a wide variety of glucosinolates, which might be involved in plant defense against fungal pathogens. In the current study first leaf of Brassica *rapa* plants, which were grown at sulfate-sufficient and sulfate-deprived conditions were infected with two necrotrophic fungi, *Alternaria brassicicola*, a specialist Brassica pathogen, and *Botrytis cinerea*, a generalist Brassica pathogen. Sulfate-deprived plants were more susceptible for the pathogenesis of the fungi than sulfur-sufficient plants. Moreover, infection of the first leaf of sulfate-sufficient plants with the fungi resulted in increase in total glucosinolate content, which was mainly due to enhanced levels of indolic glucosinolates. The increase in the glucosinolate content did not only occur in the directly infected leaf, but also in other leaves and roots, demonstrating a general systemic response to fungal infection. Though, infection of plants with *A. brassicicola* resulted in a 3-fold higher level of indolic glucosinolates than infected with *B. cinerea*. The content of 4-methoxy-indol-3-ylmethyl glucosinolate was increased to a larger extent than that of the other indolic glucosinolates. The significance of this glucosinolate in the plant resistance to fungal infection is discussed.

5.1 Introduction

Brassicaceae contain high levels of glucosinolates, sulfur-containing secondary compounds, which might have significance in plant protection against biotic stresses, *viz*. insects, fungi and herbivory (Bennett and Wallsgrove, 1994; Cole, 1997; Wittstock and Halkier, 2002; Kliebenstein, 2004; Bednarek et al., 2009; Buxdorf et al., 2013). Glucosinolates are relatively non-reactive compounds, which hardly have significance physiological functioning of plants (Rask et al., 2000). It has been assumed that they might be involved in storage of reduced sulfur in plants, though this function appears to be limited and doubtful, at least if plants are in the seedling stage (Aghajanzadeh et al., 2014). Though if plants tissue is injured, glucosinolates may be degraded by myrosinase yielding a variety of hydrolysis active products including isothiocyanate, thiocyanate, nitrile, epithionitrile or oxazolidinethione (Bones and Rossiter, 1996). The different chemical structure of glucosinolates (aliphatic, indolic and aromatic), and their derivatives (formed from glucosinolate side chain modification) and the reaction conditions (myrosinase-associated proteins, pH, metal ions) may affect the kinds of glucosinolate hydrolysis products formed by myrosinase (Halkier and Gershenzon, 2006; Kissen and Bones, 2009; Ahuja et al., 2010). It has been suggested that the glucosinolate breakdown products may be involved in plant defense response to fungal pathogens (Fenwick et al., 1983).

On the basis of their lifestyle, plant-pathogenic fungi can be classified as biotrophs and necrotrophs, which presumably induce different defense responses in the plant host (Narusakaet al., 2003; van Wees et al., 2003; Glazebrook, 2005). Biotrophic pathogenic fungi have a feeding relationship with the host plants and upon infection the host cells are not killed, whereas necrotrophic pathogenic fungi cause tissue injury and upon infection of Brassicaceae they will be exposed to glucosinolate breakdown products. It has been suggested that the degree of infection by necrotrophic fungi may be partially controlled by plant glucosinolate breakdown products (Braderet al., 2001; Tierens et al., 2001; Kliebenstein, 2004). *In vitro* experiments have shown that purified glucosinolate breakdown products had growth inhibitory effects on several necrotrophic fungispecies (Troncosoet al., 2005; Troncoso-Rojas et al., 2005; Báez-Flores et al., 2011). Moreover, the concentration of glucosinolates in leave tissue of Brassica species should be sufficient to inhibit fungal growth (Mithenet al., 1987). However, it has been suggested that necrotrophic fungi, which are specialists for Brassicaceae fungi, may have evolve defense mechanisms against the toxic glucosinolate breakdown

products (Kliebenstein, 2004). Probably not only the glucosinolate tissue content but also the glucosinolate composition may be of great importance in the plant defense against fungal pathogens. For instance mutant Arabidopsis lines which were limited in the synthesis of aliphatic glucosinolate biosynthesis appeared to be hyper susceptible to the pathogenic fungus *Sclerotinia sclerotiorum* (Stotzet al., 2011). Likewise, *Fusarium oxysporum*, was reported to be infective for Arabidopsis mutant, *gsm1*$^{-1}$, which was not able to synthesize aliphatic glucosinolates (Tierenset al., 2001). In general, indolic glucosinolates are the most common glucosinolates induced in plants upon exposure to biotic stresses. The hydrolysis of indolic glucosinolate results in the formation of an unstable glucone, which solely results in the formation of the corresponding nitrile or isothiocyanate (Hanley et al., 1985; Agerbirket al., 1998). This is in contrast with aliphatic glucosinolates, which degradation in addition to nitrile or isothiocyanate also results in the formation of thiocyanate and epithionitrile, compounds that are less toxic to the fungi (Agerbirket al., 2009). Moreover, all isothiocyanates produced from indolic glucosinolates are unstable due to a replacement of the functional group with various nucleophiles (Agerbirket al., 2009). It has been demonstrated that breakdown products of indolic glucosinolates are important for disease resistance to *Botrytis cinerea*, *S. sclerotiorum, Plectosphaerella cucumerina*and, *Phytophthora brassicae* (Sanchez-Valletet al., 2010; Schlaeppi et al., 2010; Stotz et al., 2011; Buxdorf et al., 2013). Additionally in the study with Arabidopsis it has been observed that 4-methoxy-indol-3-ylmethyl was significantly induced in response to aphids (*Myzus persicae*) feeding (Kim and Jander, 2007). This showed that modification of indol-3-ylmethyl glucosinolate was an important response to insect feeding. The model of side chain modification of indolic glucosinolates (Fig. 1) suggests that CYP81 catalyzes the first reaction in this pathway. However CYP81F2/F3 catalysis hydroxylation at position 4 of the indole ring and led to generation of 4-Hydroxy-indol-3-ylmethyl glucosinolate, CYP81F4 catalysis the formation of 1-Hydroxy-indol-3-ylmethyl glucosinolate by hydroxylation at position 1 of the indole ring. Subsequently, generation of 4-Methoxy-indol-3-ylmethyl and 1-Methoxy-indol-3-ylmethyl glucosinolate are occurred by methylation of hydroxyl group via an *O*-methyltransferase enzyme(O-MT; Hall et al., 2001; Kliebenstein et al., 2001a; Kliebenstein et al., 2001b; Pfalz et al., 2009; Pfalz et al., 2011; Frerigmann and Gigolashvili, 2014).

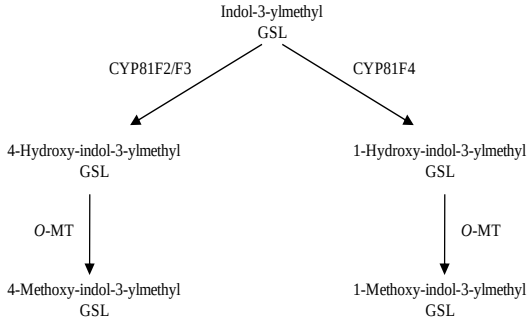

Fig. 1.The scheme of side chain modification of indol-3-ylmethyl glucosinolates.

The current study focused on the importance of glucosinolate content and composition in response of *Brassica rapa* to two necrotrophic fungi *Alternaria brassicicola*, a specialist Brassica pathogen, and *Botrytis cinerea*, a generalist Brassica pathogen. *B. rapa* was grown at sulfate-sufficient and sulfate-deprived conditions and subsequently leaves were infected with the fungi and its impact on plant response, the glucosinolate content and composition was evaluated. It was evident that both fungi had a decisive effect on the content of indolic glucosinolates.

5.2 Materials and Methods

Fungal and plant material

The freeze-dried *Alternaria brassicicola* and *Botrytis cinerea* were obtained from the CBS-KNAW Fungal Biodiversity Center (an institute of the Royal Netherlands Academy of Art and Sciences) with CBS number of 125088 and 124.58, respectively. For culturing of the fungi, freeze-dried fungi were poured into 1-2 ml of sterile water, shacked gently and kept at 22±1 ^{o}C for 8 hours. Then 200 µl of fungal suspension was transferred to specific solid agar medium in Petri dish. *Alternaria brassicicola* and *Botrytis cinerea* were grown on oatmeal agar (Bello and Epstein 2013) and hay medium agar (Atlas 1946), respectively. Then the fungi were incubated at 22± 1 ^{o}C (day) and 18± 1 ^{o}C (night) with 70-80% relative humidity and a light intensity of 300 ± 20 µmol m^{-2} s^{-1} (400-700 nm) for two weeks. Then spores were suspended in sterile water and counted in a hemocrytometer.

Seeds of *Brassica rapa* cv. Komatsuna (Van der Wal, Hoogeveen, The Netherlands) were germinated in vermiculite in a climate-controlled room. Day and night temperatures were 22 and 18 ^{o}C (± 1 ^{o}C), respectively, relative humidity was

70-80%. The photoperiod was 14 h at a photon fluence rate of 300±20 µmol $m^{-2}s^{-1}$ (400-700 nm) at plant height, supplied by Philips GreenPower LED (deep red/white 120) production modules. Ten day-old seedlings were transferred to an aerated 25 % Hoagland nutrient solution at 0.5 mM sulfate for five days and subsequently transferred to fresh Hoagland nutrient solution at 0 mM sulfate (-S, sulfate-deprived) or 0.5 mM sulfate (+S, sulfate-sufficient) in 30 l containers (twenty sets of plants per container; three plants per set). One day after transferring to the fresh Hoagland nutrient solution, the first leaf of plant was infected with two droplets of the conidial suspension. Each droplet was included 5 µl containing 2×10^8 spore ml^{-1}. Likewise, the first leaf of control plant was treated with two droplets of distils water. Three days after infection, the first and second leaf of the plant and the roots were harvested, weighed, and frozen immediately in liquid N_2 and stored at -80 °C. Leaf area (leaf surface) and lesion size were measured using an Epson Desktop Scanner and the WinRhizo software (Regents Instruments, Quebec, Canada).

Glucosinolate content

The glucosinolates content were determined by Liquid chromatography-electrospray ionization-mass spectrometry (LC/ESI-MS) method as described by (Giavaliscoet al. 2011). Frozen ground plant material (100 mg) was homogenized in 300 µl of 100% methanol and then 200 µl of chloroform for short time. Subsequently, 400 µl of water was added to the extraction and mixed for 15 s. The extract was centrifuged for 10 min at 14,000 rpm. The polar fraction was dried in the speed vacuum for at least 3 hours without heating. Next, the pellet was solved in 80% methanol on ice for 10 min. To solve the pellet completely, the suspension was sonicated for short time (5 s). The suspension was centrifuged for 15 min at 14,000 rpm at 4 °C (Tohge and Fernie 2010). 3µl of the supernatant was injected for LC/ESI-MS analysis. LC/ESI-MS was comprised an ultra-performance liquid chromatography (UPLC) system and a mass spectrometer system equipped with an electrospray ionization interface. Liquid chromatographic separation was carried out using HSS T3 C18 reversed-phase column (100 mm × 2.1 mm × 1.8µm particles), buffer A (mix water and formic acid at final concentration of 0.1% formic acid v/v) and buffer B (mix acetonitrile and formic acid at final concentration of 0.1% formic acid v/v) with flow rate 400 µl min^{-1}. Subsequent mass spectrometric analysis was performed in positive or negative ionization mode.

The capillary temperature was 250°C; the spray voltage was 3 kV with mass range 100-1500 m/z.

Statistical analysis

Data were analyzed for statistical significance using an unpaired two-tailed Student's t-test ($P < 0.01$).

5.3 Results

Susceptibility of sulfate sufficient and sulfate-deprived plants to fungal infection

B. rapa was much more susceptible to the fungus *A. brassicicola* than *B. cinerea.* Infection of the first leaf resulted in a 3-fold higher lesion area with *A. brassicicola* than that of *B. cinerea* (Fig. 2). In order to access the impact of sulfur nutrition on plant resistance, the infected *B. rapa* was exposed to sulfate deprivation for three days. Sulfate-deprivation resulted in significant increase in the size of the lesion area of the first leaf in both *A. Brassicicola* and *B. cinerea,* which was enhanced by 30% and 90%, respectively (Fig. 2).

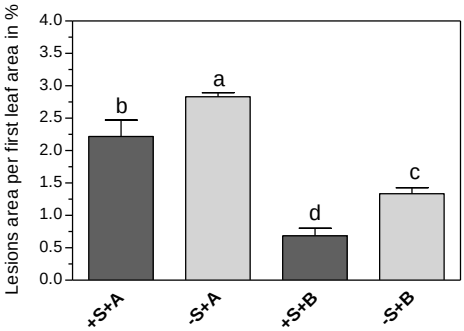

Fig. 2. Lesion size of the first leaf of *B. rapa* infected with *A. brassicicola* or *B. cinerea* at sulfate sufficient (+S) and deprived (-S) status. 10 day-old seedlings were grown on a 25 % Hoagland solution containing 0.5 mM sulfate for five days and subsequently transferred to fresh Hoagland nutrient solution at 0 mM sulfate (-S, sulfate-deprived) or 0.5 mM sulfate (+S, sulfate-sufficient). After one day, the first leaf was infected with *A. brassicicola* (+S+A and -S+A) and *B. cinerea* (+S+B and -S+B) and harvested after 3 days. Data on lesion area represent the mean of 4 measurements with 3 plants in each (± SD). Different letters indicate significant differences between treatments ($P \leq 0.01$, Student's t-test).

Content and composition of glucosinolates in sulfate-sufficient and sulfate-deprived plants

The first leaf, the second leaf and roots contained a variety of methionine-derived (aliphatic) and tryptophan-derived (indolic) glucosinolates (Table 1, Fig. 3 and 4). The roots contained the highest content of glucosinolate (mainly indolic glucosinolates), followed by second leaf and first leaf (Fig. 3). The glucosinolate content of the first leaf was 50% lower than that of the second leaf (Fig. 3).

Table 1. Nomenclature of the individual glucosinolates identified in leaves and roots of *B. rapa*.

GSL type	Trivial name	Chemical name
Aliphatic		
	Glucoerucin	4-Methylthiobutyl GSL
	Gluconapin	3-Butenyl GSL
	Progoitrin	2-Hydroxy-3-butenyl GSL
	Glucoberteroin	5-Methylthiopentyl GSL
	Glucolesquerellin	6-Methylthiohexyl GSL
Indolic		
	Glucobrassicin	Indol-3-ylmethyl GSL
	Neoglucobrassicin	1-Methoxy-indol-3-ylmethyl GSL
	4-Hydroxyglucobrassicin	4-Hydroxy-indol-3-ylmethyl GSL
	4-Methoxyglucobrassicin	4- Methoxy-indol-3-ylmethyl GSL

Despite the difference in glucosinolate content, the composition of glucosinolates of the first and the second leaf were quite similar (Fig. 4). 3-Butenyl GSL (gluconapin) and 2-hydroxy-3-butenyl GSL (progoitrin) were the major aliphatic glucosinolates present, while indol-3-ylmethyl GSL (glucobrassicin) was the major indolic glucosinolate in both the first and second leaf (Fig. 4). Moreover, total short-chain aliphatic glucosinolates (glucoerucin +gluconapin + progoitrin) almost fully formed the total aliphatic glucosinolate pool in both the first and second leaf (Fig. 5). However, composition of glucosinolates in the roots was quite different from that in the first and second leaf. In the roots, the content of short-chain aliphatic glucosinolates was almost two times higher than long-chain ones (glucoberteroin+glucolesquerellin; Fig. 5). Furthermore, 4-methylthiobutyl GSL (glucoerucin) and 5-methylthiopentyl GSL (glucoberteroin) accounted for the major proportion of aliphatic glucosinolates, whereas 4-methoxy-indol-3-ylmethyl GSL (4-methoxy-glucobrassicin) was the major indolic glucosinolate present in the roots (Fig. 4).

Sulfate deprivation resulted in strongly decreased glucosinolate content in the first and the second leaf and the roots (Fig. 3). However, content of the glucosinolates in the second leaf was more affected than that of the first leaf. Moreover, the content of the aliphatic glucosinolates was more affected than that of the indolic glucosinolates. A 4-day sulfate deprivation resulted in 75, 88 and 84 % decreases of aliphatic glucosinolate in the first leaf, the second leaf and roots, respectively, while the indolic glucosinolate content was reduced by 32, 64 and 61%, respectively (Fig. 3).

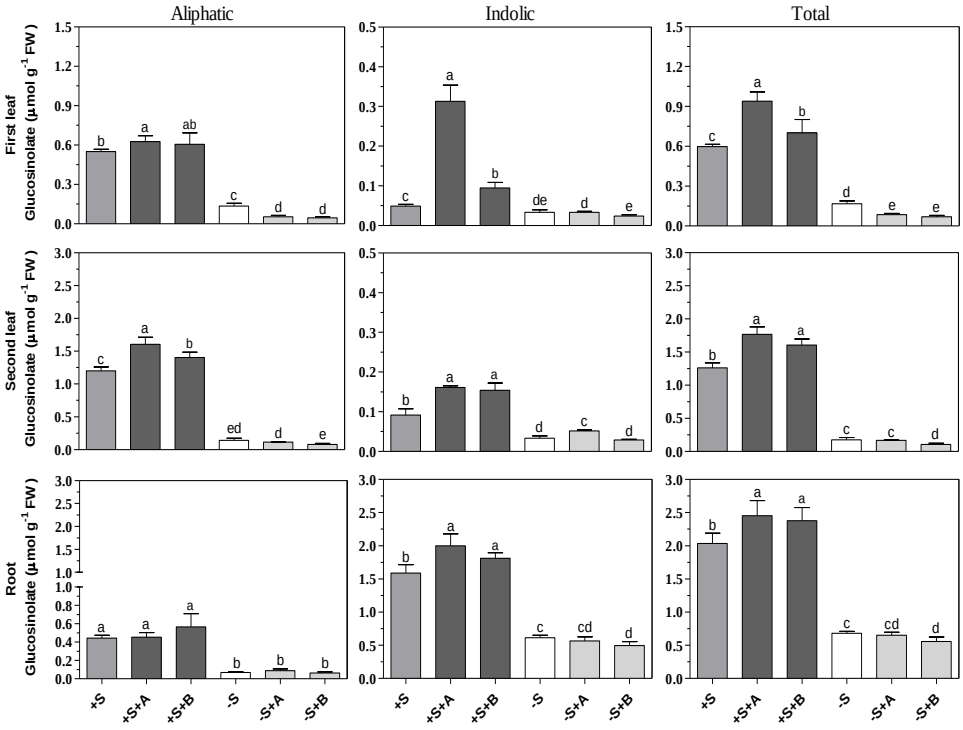

Fig. 3. Impact of *A. brassicicola* and *B. cinerea* infection on the aliphatic, indolic and total glucosinolate content of the first leaf, the second leaf and roots of *B. rapa* at sulfate-sufficient (+S) and sulfate-deprived (-S) conditions. For experimental details see legends of Fig. 2. Data on glucosinolate content represent the mean of 4 measurements with 18 plants in each (± SD). Different letters indicate significant differences between treatments ($P \leq 0.01$, Student's t-test).

Content and composition of aliphatic glucosinolates in sulfate-sufficient, sulfate-deprived and fungal-infected plants

Infection of sulfate-sufficient *B. rapa* with *A. brassicicola* and *B. cinerea* resulted in a slight but significant increase in the content of aliphatic glucosinolates in the first leaf (14%; Fig. 3). In the second leaf, infection of plants with *A. brassicicola* and *B. cinerea* resulted in 33 and 17 % increases of total aliphatic glucosinolate, respectively (Fig. 3). The biomass of the plant tissue (first and second leaf and roots) was not affected by the infection (Table 2), which showed that the enhanced aliphatic glucosinolate levels could not attributed to growth dilution. The increase in aliphatic glucosinolate content in both the first and the second leaf could fully be ascribed to an increase in the gluconapin content, a short-chain aliphatic glucosinolate (Fig. 4 and 5). The observation that the increase in the aliphatic glucosinolate content not only occurred in the first directly infected leaf, but also in the second leaf, indicated a general systemic response of the shoot to the infection. In the roots, however, the content of aliphatic glucosinolate was hardly changed after infection of the plants with both necrotrophic fungi (Fig. 3).

Infection of sulfate-deprived *B. rapa* with *A. brassicicola* and *B. cinerea* resulted in 60 and 66% reduction in the aliphatic glucosinolate content of the first leaf, respectively (Fig. 3). However, the reduction in the aliphatic glucosinolate content was not accompanied with a decrease in leaf biomass (Table 2). Furthermore, the composition of glucosinolates showed that reduction of aliphatic glucosinolate content was due to a decrease in content of gluconapin and progoitrin (Fig. 4). In the second leaf, however, the aliphatic glucosinolate content was hardly or only slightly reduced after infection of the sulfate-deprived plants with *A. brassicicola* and *B. cinerea*, respectively (Fig. 3). Similarly, the aliphatic glucosinolate content of the roots was hardly affected by infection of sulfate-deprived plants with both fungi (Fig. 3).

Content and composition of indolic glucosinolates in sulfate-sufficient, sulfate-deprived and fungal-infected plants

In contrast to the aliphatic glucosinolates, the content of indolic glucosinolates was substantially increased in plant tissue upon infection of sulfate-sufficient *B. rapa* with both fungi (Fig. 3). In the first leaf, the infection of plants with *A. brassicicola* and *B. cinerea* resulted in 6.5 and 2-fold increase in the total indolic glucosinolates content, respectively (Fig. 3). Likewise, infection of plants with both *A. brassicicola*

and *B. cinerea* resulted in a 1.7-fold increase of total indolic glucosinolates content of the second leaf (Fig. 3). However, infection of plants with both fungi only resulted in a slight increase in the indolic glucosinolates content of the roots. As infection of plants with *A. brassicicola* and *B. cinerea* resulted in a 1.3 and 1.1-fold increase of the indolic glucosinolate content of the roots, respectively (Fig. 3). In addition, the composition of indolic glucosinolates showed that the increased indolic glucosinolate content of the infected plants was for the greater part due to 4-methoxy glucobrassicin followed by glucobrassicin in the first leaf, the second leaf and roots as well (Fig. 4). Again, the increase in the indolic glucosinolates content did not only occur in the first directly infected leaf, but also in the second leaf and to a lesser extent in the roots, indicated a general systemic response of the plant to fungal infection.

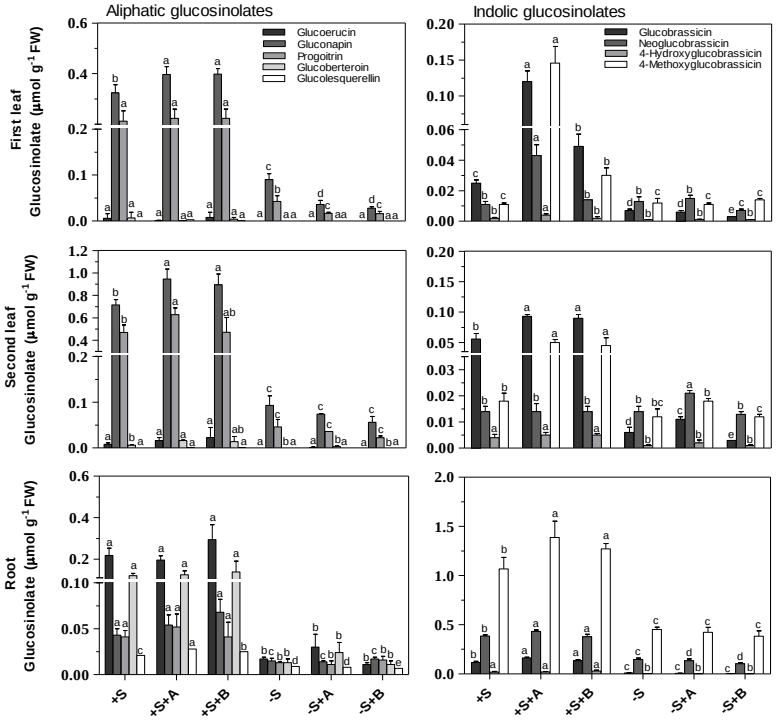

Fig. 4. Impact of *A. brassicicola* and *B. cinerea* infection on the composition of aliphatic and indolic glucosinolates of the first leaf, the second leaf and roots of sulfate-sufficient (+S) and sulfate-deprived *B. rapa* (-S). For experimental details see legends of Fig. 2. Data on glucosinolate content represent the mean of 4 measurements with 18 plants in each (± SD). Different letters indicate significant differences between treatments ($P \leq 0.01$, Student's t-test).

Infection of sulfate-deprived plants with *A. brassicicola* resulted in a slight increase in indolic glucosinolate content of the second leaf. While the indolic glucosinolate content of the first leaf and roots hardly changed. In contrast, infection of sulfate-deprived plants with *B. cinerea* resulted in slight reduction of indolic glucosinolates content of the first leaf and roots, however, that of the second leaf was hardly affected (Fig. 3).

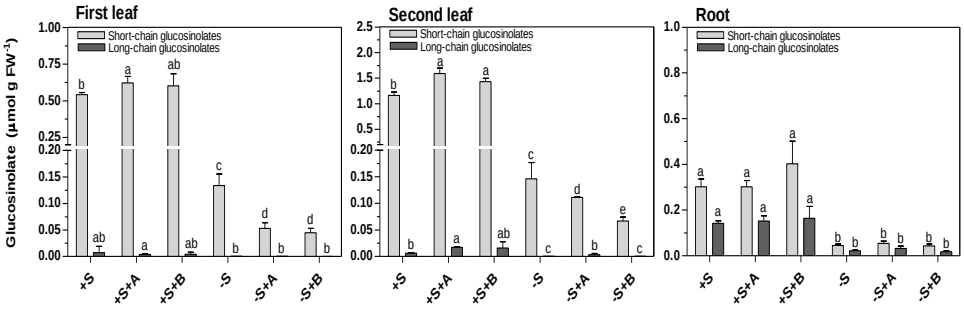

Fig. 5. Impact of *A. brassicicola* and *B. cinerea* infection on the total short-chain (glucoerucin +gluconapin+progoitrin) and long-chain (glucoberteroin+glucolesquerellin) aliphatic glucosinolates content of the first leaf, the second leaf and roots of sulfate-sufficient (+S) and sulfate-deprived *B. rapa* (-S). For experimental details see legends of Fig. 2. Data on glucosinolate content represent the mean of 4 measurements with 18 plants in each (± SD). Different letters indicate significant differences between treatments ($P \leq 0.01$, Student's t-test).

Table 2. Impact of *A. brassicicola* and *B. cinerea* infection on the biomass of the first leaf, the second leaf and roots of sulfate-sufficient (+S) and sulfate-deprived *B. rapa* (-S). Data on biomass represent the mean of 4 measurements with 18 plants in each (± SD). Different letters indicate significant differences between treatments ($P \leq 0.01$, Student's t-test).

Biomass	+S	+S +A	+S +B	-S	-S +A	-S +B
First leaf	0.347±0.010 a	0.328±0.023 a	0.342±0.031 a	0.213±0.009 b	0.189±0.023 b	0.207±0.012 b
Second leaf	0.454±0.022 a	0.446±0.019 a	0.442±0.017 a	0.228±0.016 b	0.220±0.027 b	0.261±0.023 b
Root	0.266±0.014 a	0.258±0.026 a	0.257±0.017 a	0.224±0.026 a	0.236±0.038 a	0.249±0.021 a

5.4 Discussion

The induction of glucosinolates in *B. rapa* infected with necrotrophic fungi indicated that these secondary sulfur compounds may have a positive effect on the ability of the plant to defend itself against *A. brassicicola* and *B. cinerea.* Moreover, the positive correlation between the reduction in glucosinolate content and the increased susceptibility of the sulfate-deficient plants to the fungi indicated that the compounds might function as a suppressive factor in their pathogenic response. Even plants infected with *A. brassicicola,* which is considered to be a Brassica specialist pathogen, were characterized by enhanced glucosinolates levels, not only in the leaves but also in the roots. The observation that the increase in the indolic glucosinolate content not only occurred in the first directly infected leaf, but also in the second leaf and the roots indicated a general systemic response of the plant to the fungal infection. It is assumed that specialist pathogens are able to develop different mechanisms to overcome plant defense responses. They may use mechanisms to suppress the defense responses of the host plant which prevent penetration of fungi into plant (Pedraset al., 2002; Joubertet al., 2011) and/or they may have mechanisms for detoxification of phytochemicals such as glucosinolates by converting them to less toxic derivatives (VanEttenet al., 1989; Pedras et al., 2002; Pedras et al., 2009; Pedras and Yaya, 2010; Pedras and Hossain, 2011).

Fungal infections hardly affected the aliphatic glucosinolate content of *B. rapa*, though there was a remarkable increase in indolic glucosinolate content in response to both necrotrophic fungi in infected (the first leaf) and non-infected tissues (the second leaf and roots). This observation demonstrated a stronger responsiveness of the indolic than the aliphatic glucosinolates to infection with necrotrophic fungi. This supports the suggestion that aliphatic glucosinolates contribute to a lesser extent in plant resistance to fungi (Agerbirk et al., 2009). Similarly, a defensive function of indoic glucosinolate was suggested in *Brassica napus* cultivars in response to *Alternaria brassicae* (Doughty et al., 1991). Also infection of different cultivars of *Brassica napus* with *Sclerotinia sclerotiorum* resulted in increased levels of indolic and aromatic glucosinolates (Li et al., 1999). From the current data it was evident that indolic glucosinolate 4-methoxy-indol-3-ylmethyl was induced at much higher level than the other indolic glucosinolates. A possible explanation for this finding is that fungal pathogens might induce the activity of CYP81F2/F3, the enzyme catalyzing synthesis of 4-methoxy-indol-3-ylmethyl glucosinolate to a much higher level than the enzyme CYP81F4, which is responsible for the synthesis of 1-methoxy-indol-3-ylmethyl glucosinolate. However, the synthesis of 4-hydroxy-indol-3-ylmethyl

glucosinolate, an intermediate for production of 4-methoxy-indol-3-ylmethyl from indol-3-ylmethyl glucosinolate, was hardly induced. This may indicate that 4-hydroxy-indol-3-ylmethyl glucosinolate is highly instable and quickly converts to the end product 4-methoxy-indol-3-ylmethyl glucosinolate. It has been observed that its analog, 1-hydroxy-indol-3-ylmethyl glucosinolate, is also chemically instable because of its hydroxyl group is bound to the nitrogen of the indolic ring (Fahey et al., 2001; Pfalz et al., 2009). In addition to local induction of indolic glucosinolates in the first leaf, there was also an enhanced synthesis of indolic glucosinolates upon fungal infection in the second leaf and roots. Similarly, infection of *B. rapa* with *L.maculans*, as well as infection of *B. napus* with *S. sclerotiorum* also resulted in a systemic accumulation of indolic glucosinolates (Li et al., 1999; Abdel-Faridet al., 2010). There is evidence that salicylic acid is a key modulator of systemic-acquired resistance, since its synthesis was induced upon infection of Arabidopsis with *Alternaria brassicicola* (Penninckxet al., 1996). Also exogenous application of salicylic acid to roots of *B. napus* and *B. rapa* resulted in enhanced levels of glucosinolates in their leaves (Kiddleet al., 1994; Schreiner et al., 2011). The induction of indolic glucosinolates in necrotrophic fungi-infected plant may indicate that their breakdown products have significance in protection of the plants against these pathogens. Similarly, high levels of 4-methoxy-indol-3-ylmethyl glucosinolate have also been observed in Arabidopsis plants infected with fungal pathogens and insect herbivores as well (Kim and Jander, 2007; Bednareket al., 2009). A possible metabolic pathway for indolic glucosinolate breakdown products has been suggested for antifungal defense responses (Bednareket al., 2009). 4-Methoxyindol-3-ylmethyl glucosinolate (4MI3G a fungal-induced indolic glucosinolate) would be degraded by a typical PEN2 myrosinase to an intermediate compound (4-methoxylated indol dithiocarbamate), which is formed from the isothiocyanate and a glutathione. This intermediate compound is presumed to be toxic for the fungal pathogen (Bednareket al., 2009).

The current results showed that infection of *B. rapa* by the necrotrophic fungi *A. brassicicola* and *B. cinerea* resulted in enhanced tissue levels of 4-methoxyindol-3-ylmethyl glucosinolate. There is supporting evidence that this indolic glucosinolate may have significance in plant fungal resistance. An enhancement of the tissue levels of this indolic glucosinolate by either biotechnology or breeding may contribute to an enhanced resistant of Brassicaceaea against fungi.

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